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Comparison of Serum Hepatitis C Virus RNA Concentration by Branched DNA Probe Assay With Competitive Reverse Transcription Polymerase Chain Reaction as a Predictor of Response to Interferon-α Therapy in Chronic Hepatitis C Patients

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A study was carried out to assess the correlation between the serum concentration of hepatitis C virus RNA (HCV-RNA) in patients with chronic hepatitis, as measured by competitive reverse transcription polymerase chain reaction (cRT-PCR) and branched DNA probe assay (bDNA), and response to interferon-α (IFNα) therapy. The serum HCV-RNA concentration was evaluated by both cRT-PCR and bDNA in 54 patients who had received a total dose of 480 MU of IFNα. HCV subtypes were also identified in all patients. The measurement of serum HCV-RNA concentration by bDNA correlated significantly with that of cRT-PCR. The concentration of HCV-RNA in subtype 1 patients was significantly higher than that in subtype 2 patients when measured by bDNA, but not when measured by cRT-PCR. The correlation of HCV-RNA concentration between bDNA and cRT-PCR was associated with both subtypes 1 and 2. The difference in serum HCV-RNA concentration between complete and incomplete responders was more significant when measured by bDNA probe assay than by cRT-PCR. Moreover, only 1 of 26 patients with a HCV-RNA concentration of more than 1×10^6 eq/ml as measured by bDNA probe assay attained a complete response, while 19 of 28 patients with that of less than 1×10^6 eq/ml achieved it. Measurement of serum HCV-RNA concentration by bDNA probe assay was a better predictor of clinical response to IFNα therapy than measurement by cRT-PCR.

KEY WORDS: HCV, branched DNA probe assay, competitive PCR, predictive marker, interferon therapy

INTRODUCTION

The efficacy of interferon-α (IFNα) for treatment of patients with chronic hepatitis C virus (HCV) was reported initially in 1986 [Hoofnagle et al., 1986], and is currently an established therapeutic option for this disease [Thomson et al., 1987; Davis et al., 1989; Di Bisceglie et al., 1989; Kakumu et al., 1989; Omata et al., 1989]. The response to IFNα depends on the pretreatment serum HCV-RNA concentrations and the subtype of HCV [Kanai et al., 1992; Yoshioka et al., 1992; Takada et al., 1993; Takase et al., 1993; Yamada et al., 1992; Hagiwara et al., 1993]. HCV has several subtypes [Okamoto et al., 1992; Enomoto et al., 1990; Tsukiyama-Kohara et al., 1993], and a new classification of HCV has been recently proposed [Simmonds et al., 1994]. Serum HCV-RNA concentrations have been measured with a competitive reverse transcription polymerase chain reaction assay [cRT-PCR, Hagiwara et al., 1993; Chayama et al., 1993]. The branched DNA probe assay (bDNA) was developed by Urdea et al. [1990], and is also used for HCV-RNA quantitation. The correlation of serum HCV-RNA concentrations, as measured by cRT-PCR and bDNA, and the response to IFNα therapy were studied in patients with chronic hepatitis C infection.

MATERIALS AND METHODS

Fifty-four patients with chronic hepatitis C were enrolled. The patients consisted of 37 men and 17 women with a mean age of 51.8 years. Chronic hepatitis C infection was confirmed by the presence of HCV-RNA in serum as assayed by nested RT-PCR [Shibata et al., 1991] and by liver biopsy prior to IFN therapy (Table I).

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TABLE I. Background of Patients and the Response to IFN Therapy

Characteristics	All patients	Complete responders	Incomplete responders	*P* value
Number	54	20	34	
Male: female	37: 17	15: 5	22: 12	0.619
Mean age (range)	51.8 (22–69)	49.4 (22–69)	53.2 (35–66)	0.147
Mode of transmission				
Transfusion	13	5	8	
Operation	16	5	11	
Intravenous drug users	0	0	0	
Sporadic	24	10	14	
Tattoo	0	0	0	
Acupuncture	1	0	1	
Duration of HCV carriage [median (years), range]	4.54 (0.2–32)	5.93 (0.2–32)	3.73 (0.3–16)	0.199
Biochemistry (mean ± SD)				
ALT (IU/l)	70.9 ± 49.0	64.6 ± 36.4	74.7 ± 55.2	0.468
AST (IU/l)	61.8 ± 46.6	55.0 ± 33.4	65.7 ± 52.9	0.418
Albumin (g/l)	4.14 ± 0.31	4.13 ± 0.30	4.15 ± 0.32	0.795
Globulin (g/l)	3.23 ± 0.52	3.27 ± 0.56	3.20 ± 0.50	0.677
Alkaline phosphatase (IU/l)	186.3 ± 67.1	183.5 ± 65.9	188.0 ± 68.8	0.811
γ-GTP (mU/ml)	45.5 ± 38.0	39.5 ± 39.1	49.0 ± 37.5	0.382
Total bilirubin (mg/dl)	0.72 ± 0.27	0.62 ± 0.20	0.78 ± 0.29	0.273
Serology (on admission)				
Anti-HCV+ (2nd generation)	54	20	34	
HCV-RNA+ (nested PCR)	54	20	34	
Subtype				
1a	0	0	0	
1b	34	9	25	
2a	20	11	9	0.0360
2b	0	0	0	
HCV-RNA concentration (competitive PCR)	5.34 ± 1.04	4.98 ± 0.92	5.56 ± 1.06	0.0321
HCV-RNA concentration (branched-DNA probe assay)[a]	4.33 ± 7.47	0.76 ± 0.98	6.93 ± 8.76	<0.0001
Histology				
Chronic persistent hepatitis	9	2	7	
Chronic active hepatitis-2A	29	14	15	0.182
Chronic active hepatitis-2B	16	4	12	

[a]Considering HCV concentrations of bDNA probe assay undetectable cases (under 0.5 Meq/ml) as being equal to 0.5 Meq/ml.

Each patient received 480 MU of lymphoblastoid IFNα (Sumiferon; Sumitomo Pharmaceutical Ltd., Osaka, Japan). IFNα was administered daily for the first 2 weeks and then three times a week for the following 22 weeks in all 54 patients. The HCV subtype was determined by Okamoto et al.'s method [1992], and was identified as 1a, 1b, 2a, and 2b using the more recent nomenclature [Simmonds et al., 1994].

Serum HCV-RNA concentrations were measured by both cRT-PCR and bDNA prior to the initiation of IFNα therapy [Wang et al., 1989; Gilliland et al., 1990; Hagiwara et al., 1993; Chayama et al., 1993; Sanchez-Pescador et al., 1992; Lau et al., 1993; Sherman et al., 1993]. During cRT-PCR, 2 μl of cDNA were coamplified with a fixed amount of pUHC101Δ(10^1-10^6), the competitive template, and the amount of cDNA in the sample was estimated as the standard for every assay [Chayama et al., 1993]. The bDNA signal amplification assay (Quantiplex™ HCV-RNA, version 1.0, Chiron Corporation, Emeryville, CA) was also used [Lau et al., 1993; Urdea, 1993].

The presence of HCV-RNA was assessed by the nested PCR just prior to therapy, at the end of the therapy, and at 6 months after the cessation of the therapy. Complete responders were defined as patients showing clearance of HCV-RNA both at the end of the therapy and at 6 months after the cessation of the therapy. Those who did not clear HCV-RNA were classified as incomplete responders.

Statistical analyses were carried out using Pearson's test, the chi-square test, and Mann-Whitney's rank sum test where appropriate. A P value <0.05 was considered significant.

The entire protocol was approved by the hospital ethics committee and carried out in compliance with the Helsinki declaration.

RESULTS

Of the 54 patients, 34 were classified as subtype 1b and 20 as subtype 2a. Serum HCV-RNA was detected in all patients by cRT-PCR and the mean HCV-RNA concentration was 5.38 ± 1.05 log (copies)/ml (Table I). Serum HCV-RNA was undetectable in 23 (42.6%) patients by the bDNA probe assay, which did not detect concentrations below 0.5×10^6 eq/ml. The mean concentration of HCV-RNA was $7.17 \pm 8.89 \times 10^6$ eq/ml in the other 31 patients.

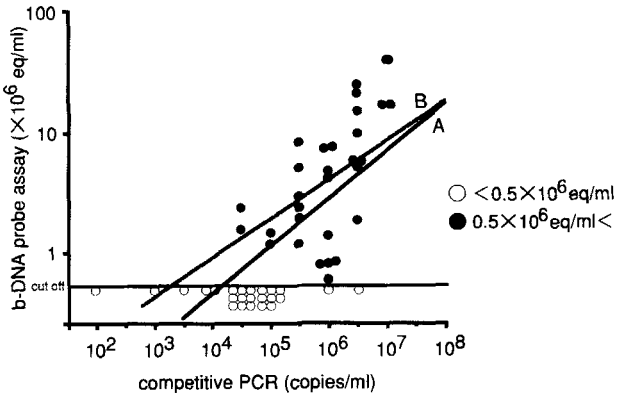

Fig. 1. Correlation between serum HCV-RNA concentrations determined by competitive PCR and by bDNA probe assay. A, Correlation fit when the serum HCV-RNA concentration was considered to be 0.5×10^6 eq/ml in patients with undetectable HCV-RNA by bDNA probe assay. B, Correlation fit when patients with undetectable HCV-RNA by bDNA probe assay were removed.

Correlation Between Serum HCV-RNA Concentrations as Detected by cRT-PCR and by the bDNA Probe Assay

There was a significant correlation between serum HCV-RNA concentrations measured by cRT-PCR and by the bDNA probe assay. The correlation coefficient was 0.6089 ($P = 0.0003$) when patients with undetectable serum HCV-RNA by the bDNA probe assay were removed. The correlation coefficient was 0.7009 ($P < 0.0001$) when the HCV-RNA concentration was considered to be 0.5×10^6 eq/ml in patients with serum HCV-RNA undetectable by the bDNA probe assay (Fig. 1). There was a significant difference in serum HCV-RNA concentrations as measured by the bDNA probe assay between patients with subtype 1b and those with subtype 2a. When the serum HCV-RNA concentration was considered to be 0.5×10^6 eq/ml in patients with undetectable HCV-RNA, subtype 1b patients had a mean serum HCV-RNA concentration of $6.19 \pm 8.89 \times 10^6$ eq/ml and subtype 2a patients had a mean concentration of $1.16 \pm 1.31 \times 10^6$ eq/ml ($P = 0.0154$). There was no significant difference when HCV-RNA was measured by cRT-PCR. The mean serum HCV-RNA concentration was 5.44 ± 1.09 log (copies)/ml in subtype 1b patients and 5.18 ± 0.95 log (copies)/ml in subtype 2a patients ($P = 0.1846$).

There was a significant correlation between serum HCV-RNA concentrations in patients with specific subtypes as measured by cRT-PCR and by the bDNA (Fig. 2). When patients with undetectable serum HCV-RNA by the bDNA probe assay were included with a concentration of 0.5×10^6 eq/ml, the correlation coefficient was 0.7813 ($P < 0.0001$) in subtype 1b patients and was 0.5235 ($P = 0.0178$) in subtype 2a patients. There was no significant difference between the correlation coefficients of subtype 1b and 2a patients.

Evaluation of Response to IFNα Therapy

Twenty patients were complete responders and 34 were incomplete responders (Table I). There were no significant differences between these two types with regard to age, sex, duration of disease, mode of transmission, histology, and alanine aminotransferase (ALT), asparate aminotransferase (AST), albumin, globulin, alkaline phosphatase (ALP), total bilirubin, and gamma glutamyl transpeptidase (γ-GTP) concentrations before IFNα therapy. There were significant differences between complete and incomplete responders in HCV subtype ($P = 0.0360$) and HCV-RNA levels as measured by cRT-PCR ($P = 0.0240$) and by the bDNA ($P < 0.0001$).

The percentage of complete responders decreased with increasing serum HCV-RNA concentrations, and only 3.85% of patients with a serum HCV-RNA concentration greater than 1.0×10^6 eq/ml by bDNA probe assay achieved a complete response (Table II). The serum HCV-RNA concentration measured by bDNA prior to IFNα therapy was a better predictor for complete response to IFNα therapy ($P < 0.0001$) than the concentration measured by cRT-PCR ($P = 0.1798$).

DISCUSSION

The serum HCV-RNA concentration is an important factor for studying the natural history of chronic hepatitis C and for predicting the response to IFNα therapy. Before the development of the bDNA assay as a method of HCV quantitation, cRT-PCR was the mainstay of evaluation [Yamada et al., 1992; Hagiwara et al., 1993; Chayama et al., 1993]. However, quantitative analysis by PCR requires special expertise and is too expensive for clinical use. In addition, quantitation of serum HCV-RNA by cRT-PCR may give incorrect results because of the marked amplification of target nucleic acids.

bDNA signal amplification was developed by Urdea et al. [1990]. It is used to assay the serum HCV-RNA concentrations [Sanchez-Pescador et al., 1992], and is available commercially in some countries. The assay is based on the specific hybridisation of synthetic oligonucleotides (target probes) to the 5′ noncoding and core regions of HCV-RNA in a crude proteinase K-SDS-treated serum extract. The signal is proportional to the level of target nucleic acid since the target is not amplified, thereby avoiding quantitation errors.

In the present study, serum HCV-RNA concentrations measured by cRT-PCR correlated with those obtained by bDNA probe assay, although the HCV-RNA concentrations in some patients as measured by the bDNA probe assay were under 0.5×10^6 eq/ml, despite a PCR-derived concentration of more than 10^5 copies/ml. Serum HCV-RNA concentrations in patients with subtype 1b were significantly higher than those in patients with subtype 2a, although there was no significant difference between the correlation coefficients. This suggests that the serum HCV-RNA concentrations in patients with subtype 2a may be underestimated by the bDNA probe assay. The serum HCV-RNA concentration is an im-

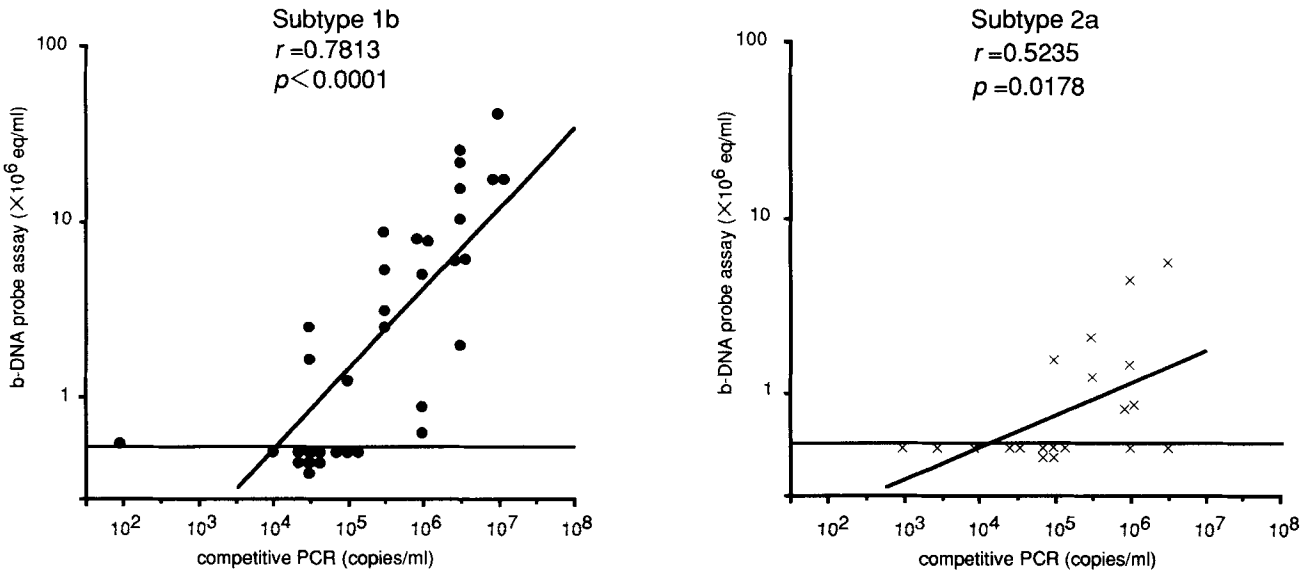

Fig. 2. Correlation between serum HCV-RNA concentrations as measured by competitive PCR and by bDNA probe assay among patients with HCV subtypes 1b and 2a.

TABLE II. Rate of Complete Responders According to HCV-RNA Level, as Measured by cPCR and by the bDNA Probe Assay

bDNA probe assay ($\times 10^6$ eq/ml)	cPCR (copies/ml) $<10^4$	$10^4 \leqq <10^6$	$10^6 \leqq$	Total	
<1	3/4 (75.0%)	11/18 (61.1%)	5/6 (83.3%)	19/28 (67.9%)	
1 ≦ <10	0/0 (0.0%)	0/10 (0.0%)	1/9 (11.1%)	1/19 (5.3%)	*
10≦	0/0 (0.0%)	0/0 (0.0%)	0/7 (0.0%)	0/7 (0.0%)	
Total	3/4 (75.0%)	11/28 (39.3%)	6/22 (27.3%)		

*$P < 0.0001$.
**$P = 0.1798$.

portant predictor of response to IFNα therapy, and a low HCV-RNA concentration suggests a marked likelihood of achieving a complete response [Yamada et al., 1992; Hagiwara et al., 1993; Chayama et al., 1993]. However, HCV-RNA quantitation by cRT-PCR may not provide clear prognostic information; the prediction of a complete response in patients with an intermediate or high HCV-RNA concentration (4–6 or >6 log [copies]/ml) is unclear, while the clinical course in patients with a low concentration (less than 4 log [copies]/ml) is more certain (Fig. 3).

Evaluation of HCV-RNA by the bDNA assay distinguishes more clearly between complete and incomplete responders. The likelihood of a complete response is very low in patients with serum HCV-RNA concentrations greater than 1.0×10^6 eq/ml. This finding remains in patients with intermediate or high serum HCV-RNA concentrations, as measured by cRT-PCR. The serum HCV-RNA concentration by the bDNA assay is less than 1.0×10^6 eq/ml in some patients with intermediate or high HCV-RNA concentrations by cRT-PCR, and the likelihood of a complete response is also high. Therefore, the serum HCV-RNA concentration measured by the bDNA assay has a higher predictive value than measurement by cRT-PCR in determining the possibility of a complete response.

HCV subtype is thought to affect the response to IFNα treatment regardless of serum HCV-RNA concentrations when evaluated by cRT-PCR [Tsubota et al., 1994]. However, the effect of viral subtype on the response to therapy declines when the HCV-RNA concentration is measured by the bDNA assay. In the present study there was no significant difference between the number of patients with subtype 1b and subtype 2a who achieved complete response ($P = 0.4183$) when focusing on the patients with HCV-RNA levels under 1.0×10^6 eq/ml. This fact is supported by the report which indicates that the patients with subtype 1b have a similar complete and sustained response rate compared to subtype 2a when the HCV-RNA concentrations are stratified into greater or less than 1.0×10^6 eq/ml [Orito et al., 1994]. The predictive value of the serum HCV-RNA concentration includes the effect of the HCV subtype when it is evaluated by the bDNA probe method, increasing the value of HCV-RNA quantitation by bDNA. The predictive significance of the HCV subtype, independent

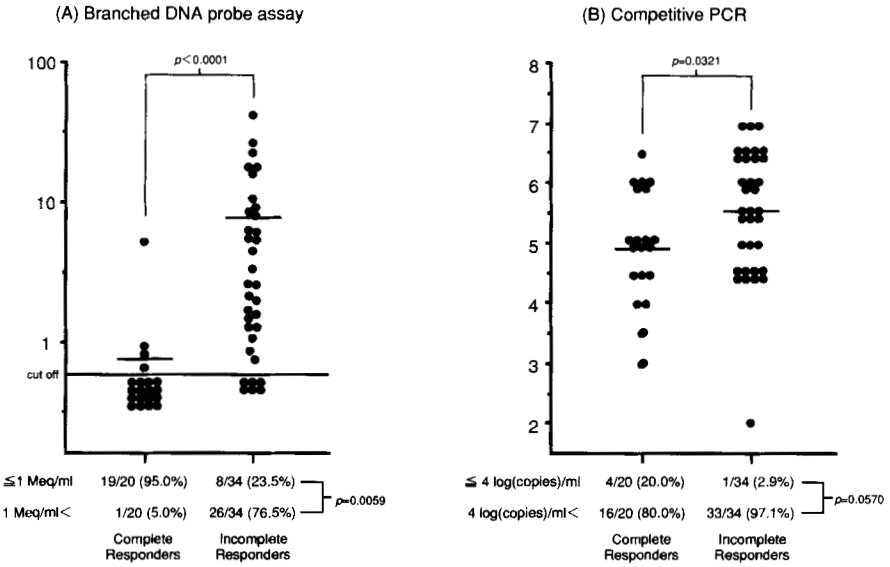

Fig. 3. Serum HCV-RNA concentration as measured by bDNA probe assay **(A)** and competitive PCR **(B)** according to response to IFNα therapy.

of the serum HCV-RNA concentration, has not been determined and further research is required in this area.

This study suggests that measurement of serum HCV-RNA concentration by the bDNA probe assay is a better predictor of a complete response to IFNα therapy for chronic HCV patients than measurement by cRT-PCR, although the bDNA probe assay is less sensitive than cRT-PCR for detecting HCV-RNA at lower concentrations [Sanchez-Pescador et al., 1992].

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